Separation of Products of Thebaine Rearrangement by Capillary Electrophoresis in the Presence of Cyclodextrins

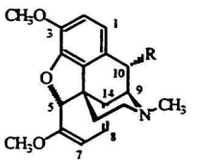
B. PROKSA

Slovakofarma a.s., SK-920 27 Hlohovec
e-mail: proksab@slofa.sk

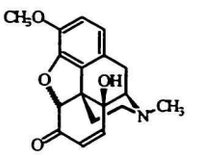
Received 6 April 2000

Separation of thebaine and products of its oxidation (14-hydroxycodeinone, 14-hydroxycodeinone *N*-oxide, (10*S*)-hydroxythebaine, (8*S*)-hydroxyoxycodone) as well as rearrangement in acidic media (thebenine, morphothebaine) was studied by capillary zone electrophoresis in the presence of modified cyclodextrins. Conditions for separation of thebaine/10-hydroxythebaine were compared with those of morphine/10-hydroxymorphine and codeine/10-hydroxycodeine.

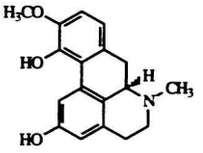
Cyclodextrins, cyclic oligosaccharides forming complexes with organic compounds have been used as media modifiers in various analytical techniques, *e.g.* NMR, circular dichroism, HPLC, GLC or capillary electrophoresis and they have been especially useful for analysis of structural, positional or stereo isomers [1—5]. Specific separations of analytes and intramolecular interactions are based on noncovalent effects, *e.g.* electrostatic interactions, formation of hydrogen bonds, hydrophobicity, release of conformation strain, van der Waals forces, *etc.* [6]. Capillary zone electrophoresis (CZE) in the presence of cyclodextrins



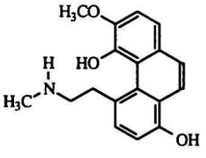
I R = H
VI R = OH



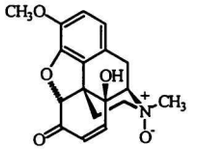
II



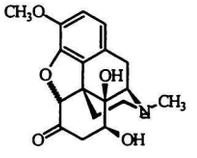
III



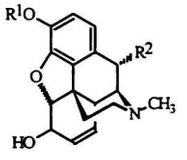
IV



V



VII



VIII $R^1 = R^2 = H$
IX $R^1 = H$ $R^2 = OH$
X $R^1 = CH_3$ $R^2 = H$
XI $R^1 = CH_3$ $R^2 = OH$

was applied for analysis of alkaloids of *Papaver somniferum* L. in various matrices, *e.g.* in pharmaceutical formulations [7, 8] or in samples of illicit drugs [9, 10].

This paper deals with separation of products of thebaine rearrangement by capillary zone electrophoresis in the presence of cyclodextrins.

P. somniferum biosynthesizes an array of structurally different alkaloids, some of them are used in form of salts with inorganic acids, the other are the starting material for semisynthetic variation to the more potent drugs. Thebaine (*I*) (see the formulas) is the representative of the latter group. Oxidation of the base *I* with hydrogen peroxide in neutral or mild alkaline solutions gave a mixture of diastereomeric *N*-oxides; their separation was described recently [11]. This reaction performed in an organic acid as a solvent spiked with mineral one afforded 14-hydroxycodeinone (*II*) [12]. However, base *I* is much more sensitive to acidic media than the other morphinane alkaloids; according to reaction conditions morphothebaine (*III*) or thebenine (*IV*) are formed [13, 14]. Capillary zone electrophoresis in a background electrolyte (BGE) completed with β-cyclodextrin (B-CD) proved to be a useful tool to monitor this transformation. Only a simple sample preparation was necessary for this assay, measurement took only a few minutes and the separation of peaks was satisfactory. Thebaine transformation is not a simple reaction, it proceeds *via* a series of intermediates, many of them are unidentified [15]. On the other hand, it is a quick reaction (less than 5 min) when performed in boiling solvents (Figs. 1—3).

CZE was used in analysis of reaction mixtures during oxidative transformation of *I* to *II* where also 14-hydroxycodeinone *N*-oxide (*V*), (10*S*)-hydroxythebaine (*VI*), and (8*S*)-hydroxyoxycodone (*VII*) were identified as by-products [16]. *N*-Oxide *V*, the main impurity, was well separated from the parent compound *II* and all other alkaloidal constituents of reaction mixture due to its low mobility. However, separation of compounds *I*, *II*, *V*—*VII* was sensitive to composition of BGE and it was possible in the presence of cyclodextrins only (Figs. 4 and 5). Remarkable was the high specific affinity of *II* to B-CD and its methylated derivative DB-CD in comparison with the other analyzed, structurally similar compounds. γ-Cyclodextrin (G-CD) was used in the further experiments because the balanced ratio of migration times and resolution of peaks of studied compounds was attained in its presence. Resolution of adjacent peaks was dependent on the concentration of added selector; it proportionally increased with concentration in pair of peaks of *I*/*V* while rapidly decreased with the pair *II*/*VII*. Concentration 7.0 mmol dm^{-3} of G-CD was chosen as a compromise between migration times and resolution when all peaks were resolved down to the baseline (Fig. 6).

Morphinane alkaloids undergo decomposition, mainly oxidation in course of ripening of the poppy or during storage in solutions or in solid state after

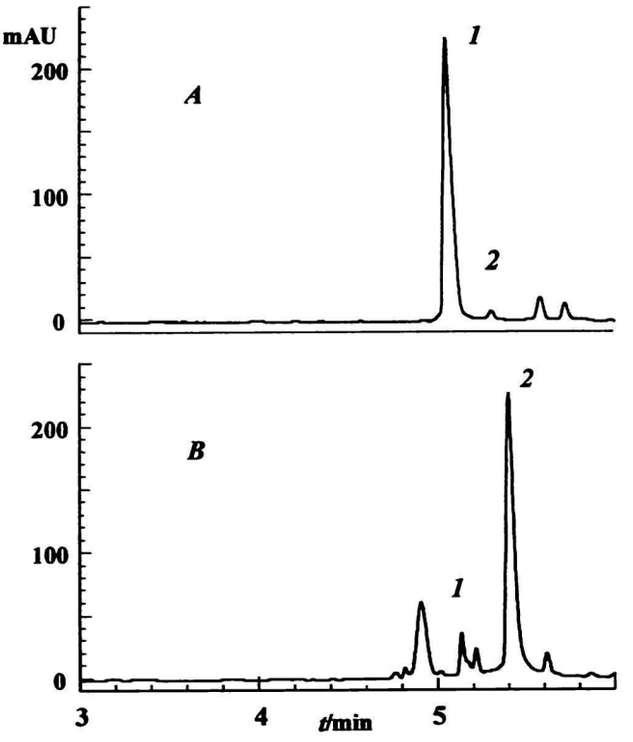


Fig. 1. Electrophoregrams of reaction mixture of thebaine (*I*) rearrangement to thebenine (*IV*) in boiling aqueous HCl (0.3 M). *A*: 1 min, *B*: 5 min. Capillary: 40 cm × 50 mm, 100 × 10^{-3} M-TRIS/phosphate, pH 2.8, 30 kV, 25 °C, 10 × 10^{-3} M-B-CD. *1*. Thebaine (*I*), *2*. thebenine (*IV*).

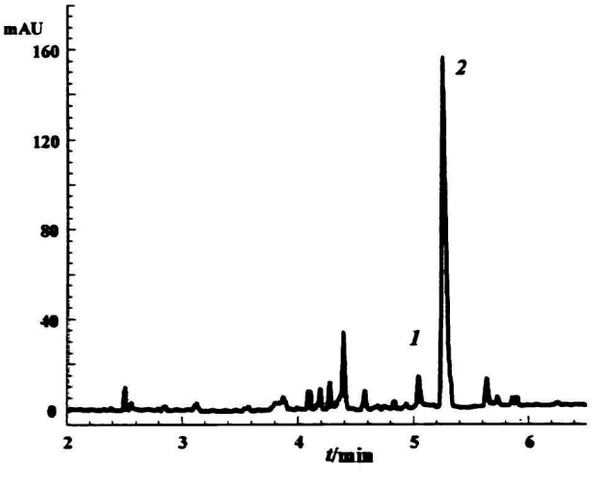


Fig. 2. Transformation of thebaine (*I*) to morphothebaine (*III*) after 5 min boiling in aqueous HCl (5.5 M). Capillary: 40 cm × 50 mm, 100 × 10^{-3} M-TRIS/phosphate, pH 2.8, 30 kV, 25 °C, 10 × 10^{-3} M-B-CD. *1*. Thebaine (*I*), *2*. morphothebaine (*III*).

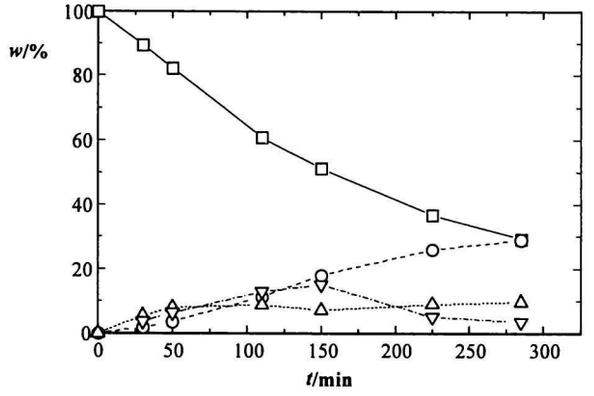


Fig. 3. The time dependence of concentration of thebaine (*I*) during its transformation to thebenine (*IV*) in 0.3 M aqueous HCl at 25 °C (*Z*-1, *Z*-2 – unknown intermediates). □ *I*, ○ *IV*, △ *Z*-1, ▽ *Z*-2.

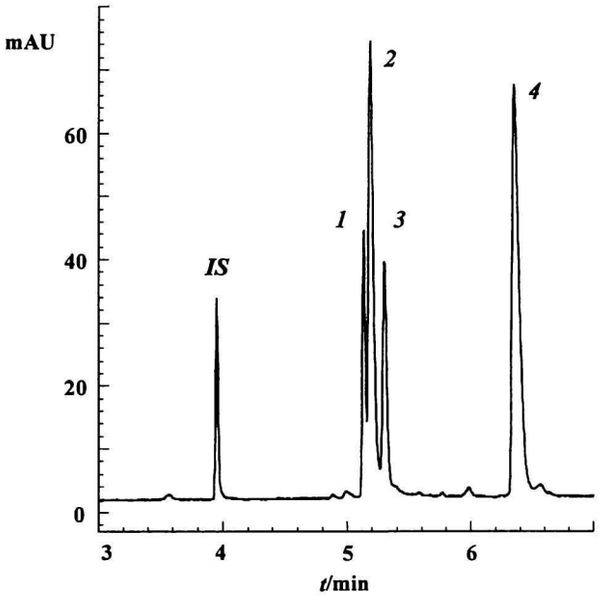


Fig. 4. Separation of by-products of thebaine oxidation by CZE. IS = pyridoxine (marker), *1.* thebaine (*I*), *2.* (10*S*)-hydroxythebaine (*VI*), *3.* (8*S*)-hydroxyoxycodone (*VII*), *4.* 14-hydroxycodeinone (*II*). Capillary: 40 cm × 50 mm, 100 × 10^{-3} M-TRIS/phosphate, pH 2.8, 30 kV, 25 °C, 5 × 10^{-3} M-B-CD.

their isolation. The most susceptible positions for oxidation of morphine (*VIII*) are C-2, C-6, C-10, and C-17, forming thus pseudomorphine, morphinone, 10-hydroxymorphine, 10-oxomorphine or morphine *N*-oxide, respectively. Pseudomorphine and morphine *N*-oxide are the first indicators of *VIII* degradation in aqueous solutions [17], (10*S*)-hydroxymorphine (*IX*) was identified in samples of solid morphine sulfate [18]. It was interesting to compare separation of the genuine alkaloids *I*, *VIII*, and *X* with their 10-hydroxy derivatives *VI*, *IX*, *XI* by CZE.

All three pairs, *I*/*VI*, *VIII*/*IX*, and *X*/*XI*, were resolved by CZE in a plain BGE. While the resolution of peaks of pairs *VIII*/*IX* and *X*/*XI* was very high and almost of the same value at pH 2.8 and 5.4, that one of thebaine/10-hydroxythebaine (*I*/*VI*) was substantially lower in both electrolytes. Electrophoretic mobility, at the constant electric field and also the terminal velocity of an ion is indirectly proportional to the radius of spherical particle [19]. Studied compounds differ in radius and therefore also in molar volume. Calculated molar volume of studied compounds increased from *VIII* to *VI* (Table 1), but the migration times of these alkaloids were not proportional to their calculated volumes. In this calculation hydration of molecules was not considered, which is apparently the highest with *IX* and the lowest with *I* and con-

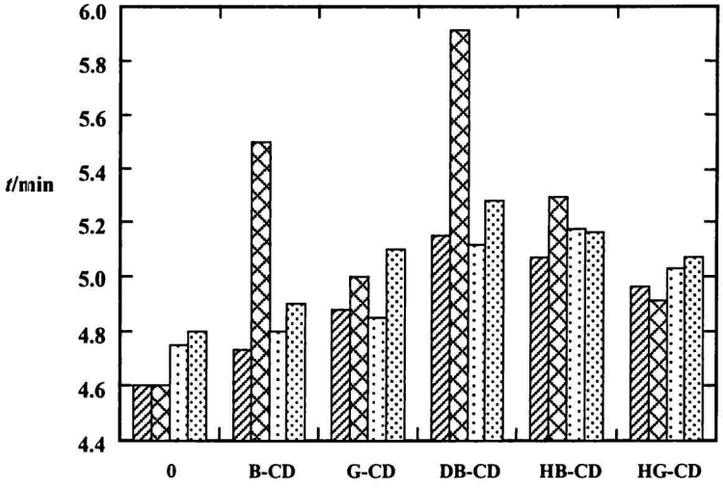


Fig. 5. Migration times of compounds *I*, *II*, *VI*, and *VII* in the presence of cyclodextrins. Capillary: 40 cm × 50 mm, 100 × 10^{-3} M-TRIS/phosphate, pH 2.8, 30 kV, 25 °C, 5 × 10^{-3} M-CD. ▨ *I*, ▫ *II*, ▫ *VI*, ▫ *VII*.

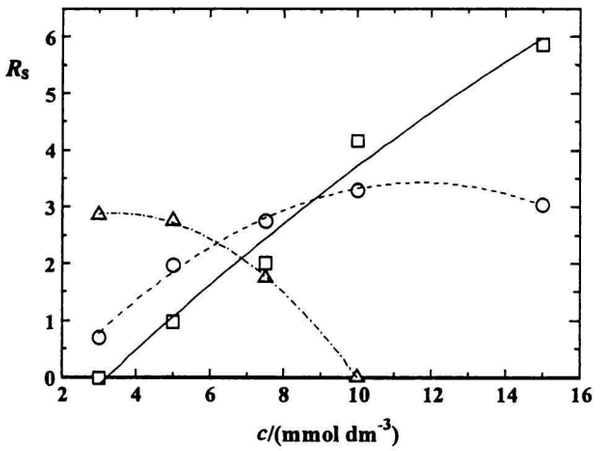


Fig. 6. Dependence of resolution of peaks of compounds *I*, *II*, *VI*, and *VII* on concentration of G-CD. Capillary: 40 cm × 50 mm, 100 × 10^{-3} M-TRIS/phosphate, pH 2.8, 30 kV, 25 °C. □ *I*/*VI*, ○ *I*/*II*, △ *II*/*VII*.

sequently the actual volume of hydrated molecules is other than the calculated one. This assumption might be used to explain differences in the observed velocities of studied compounds.

Higher migration times were observed due to specific interactions of guest molecules with the host ones after addition of cyclodextrins to BGE (Figs. 7 and 8). Ionizable carboxymethylated cyclodextrins CD (CB-CD and CG-CD) at pH below their pK_a behave like a stationary phase, at higher pH they move towards anode. Resolution of relevant peaks with increasing concentration of CB-CD reached a minimum at c = 3, 5, 7 mmol dm^{-3} for pairs *I*/*VI*, *X*/*XI*, and *VIII*/*IX*, respectively (Fig. 9). Below these critical concentrations the peaks of native alkaloids migrated with higher velocity than their oxidized derivatives; above this concentration they moved slower. While migration velocity of 10-hydroxy derivatives changed only very little with increasing concentration of CB-CD, the native alkaloids formed much stronger complexes with the host molecule, which resulted in their lower migration. Therefore, change in the concentration of added selector may be used for fine tuning of separation of morphinane alkaloids.

Table 1. Migration Time (t), Calculated Values of Molar Volume (V) and Hydrophilic Surface (HS) of Studied Compounds

Compound	t	V	HS
	min	cm^3 mol^{-1}	%
I	4.60	175.2	73.3
VI	4.75	180.9	78.1
VIII	4.65	155.6	82.7
IX	5.05	161.4	90.8
X	4.61	165.6	76.9
XI	4.00	171.4	81.2

Capillary: 40 cm × 50 mm, 0.1 M-TRIS/phosphate, pH 2.8, 30 kV, 25 °C.

EXPERIMENTAL

β-Cyclodextrin (B-CD), 2-hydroxypropyl-β-cyclodextrin (HB-CD), 2-hydroxypropyl-γ-cyclodextrin (HG-CD), γ-cyclodextrin (G-CD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DB-CD), carboxymethylated β-cyclodextrin (CB-CD), carboxymethylated γ-cyclodextrin (CG-CD) were purchased from Cyclolab (Budapest). All other chemicals were of anal. grade.

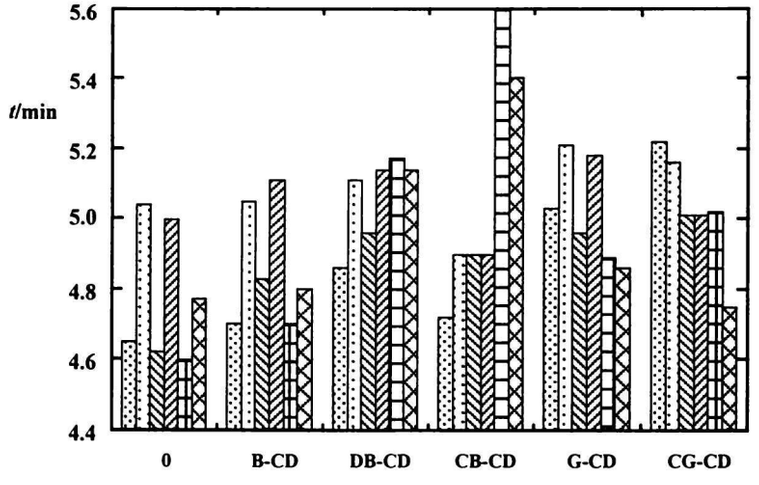


Fig. 7. Migration times t of thebaine (*I*), morphine (*VIII*), codeine (*X*), and their 10-hydroxy derivatives *VI*, *IX*, and *XI*. Capillary: 40 cm × 50 mm, 100 × 10^{-3} M-TRIS/phosphate, pH 2.8, 30 kV, 25 °C, 5.0 × 10^{-3} M-CD. ◪ *VIII*, ◘ *IX*, ◙ *X*, ◪ *XI*, ◘ *I*, ◙ *IV*.

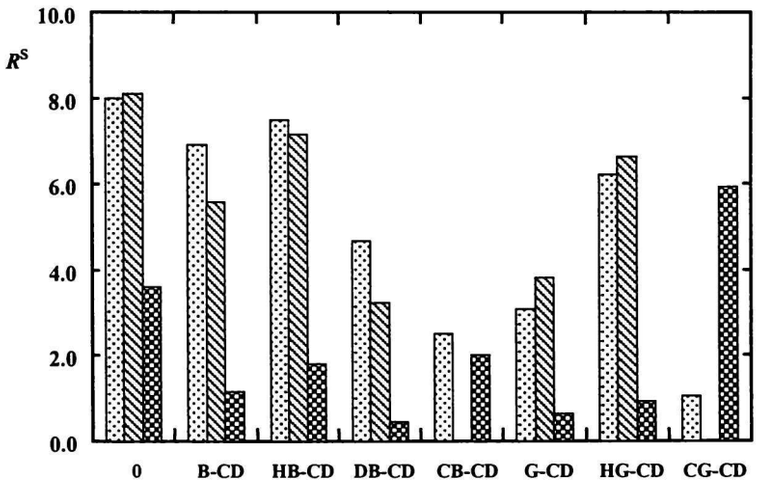


Fig. 8. Resolution of peaks of morphinane alkaloids *I*, *VIII*, *X* and their (10*S*)-hydroxy derivatives *VI*, *IX*, and *XI* in the presence of cyclodextrins. Capillary: 40 cm × 50 mm, 100 × 10^{-3} M-TRIS/phosphate, pH 2.8, 30 kV, 25 °C, 5.0 × 10^{-3} M-CD. ⊡ *VIII/IX*, ⊠ *X/XI*, ◘ *I/VI*.

HP ^{3D}Capillary Electrophoresis apparatus (Hewlett—Packard, Waldbronn, Germany) was used for analysis with an untreated fused silica capillary 48.5 cm × 0.05 mm (effective length 40 cm) and extended light path (× 3). Prior to use, the bare silica capillary was rinsed with 1 M-NaOH (15 min), distilled water (10 min), and the BGE (0.1 M-TRIS/phosphate, pH 2.8, 5 min). Between analyses the capillary was flushed with 0.01 M phosphoric acid (1.0 min), distilled water (0.5 min), and buffer solution (2.0 min). Samples were pressure-injected at 50 kPa for 2 s.

Molar volume of studied compounds was calculated with the program Molecular Modeling Pro (Version 1.44, WindowChem Software, Fairfield, USA).

Preparation of Samples for Capillary Zone Electrophoresis

Sample (5 mg) or 0.1 cm^3 of reaction mixture was dissolved in 2 cm^3 of 0.1 M aqueous HCl, solution was diluted to 10 cm^3 with background electrolyte (0.1 M-TRIS/phosphate buffer, pH 2.8 with addition of appropriate cyclodextrin) and before pressure injection (100 kPa s) it was filtered through a 0.20 μm nylon membrane filter.

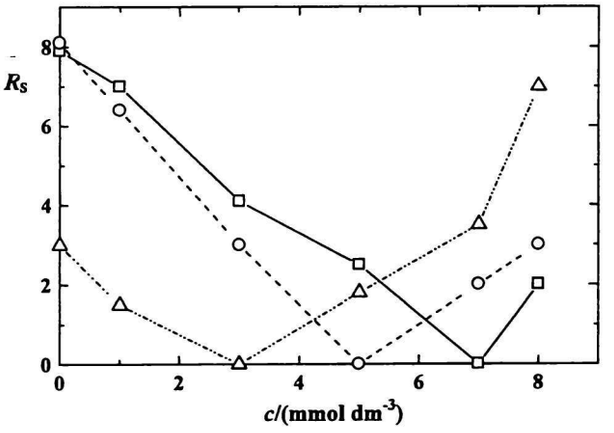


Fig. 9. Dependence of resolution (R_S) of thebaine (*I*), morphine (*VIII*), codeine (*X*) and their (10*S*)-hydroxy derivatives *VI*, *IX*, *XI*, respectively, on concentration of CB-CD. Capillary: 40 cm × 50 mm, 100 × 10^{-3} M-TRIS/phosphate, pH 2.8, 30 kV, 25 °C. □ *VIII/IX*, ○ *X/XI*, △ *I/VI*.

REFERENCES

1. Liu, L. and Nussbaum, M. A., *J. Pharm. Biomed. Anal. 19*, 679 (1999).
2. Hadley, M. R., Gabriac, S. D., and Hutt, A. J., *Chirality 11*, 409 (1999).
3. Lipkowitz, K. B. and Stoehr, C. M., *Chirality 8*, 341 (1996).
4. Felli, C., Carrea, G., Chiari, M., De Amici, M., and De Micheli, C., *J. Chromatogr., A 741*, 287 (1996).
5. Thunhorst, M., Otte, Y., Jefferies, T. M., Branch, S. K., and Holzgrabe, U., *J. Chromatogr., A 818*, 239 (1998).
6. Connors, K. A., *Chem. Rev. 97*, 1325 (1997).
7. Bjørnsdottir, I. and Hansen, S. H., *J. Pharm. Biomed. Anal. 15*, 1083 (1997).
8. Proksa, B., *J. Pharm. Biomed. Anal. 20*, 179 (1999).
9. Gong, Z., Zhang, Y., Zhang, H., and Cheng, J., *J. Chromatogr., A 855*, 329 (1999).
10. Chiarotti, M. and Fucci, N., *J. Chromatogr., B: Biomed. Sci. Appl. 733*, 127 (1999).
11. Proksa, B., *J. Chromatogr., A 818*, 251 (1998).
12. Krassnig, R., Hederer, Ch., and Schmidhammer, H., *Arch. Pharm. Pharm. Med. Chem. 329*, 325 (1996).
13. Fleischhacker, W., *Monatsh. Chem. 99*, 1568 (1968).
14. Channon, R. T., Kirby, G. W., and Massey, S. R., *Chem. Commun. 1969*, 92.

15. Bentley, K. W., in *The Alkaloids*, Vol. *13*. (Manske, R. H. F., Editor.) P. 13. Academic Press, New York, 1971.
16. Proksa, B., *Arch. Pharm. Pharm. Med. Chem. 332*, 369 (1999).
17. Yeh, S.-Y. and Lach, J. L., *J. Pharm. Sci. 50*, 30 (1961).
18. Farsam, H., Eiger, S., Lameh, J., Rezvani, A., Gibson, B. W., and Sadee, W., *Pharm. Res. 7*, 1205 (1990).
19. Fu, S. and Lucy, C. A., *Anal. Chem. 70*, 173 (1998).